Microalgae as source of bioactive peptides

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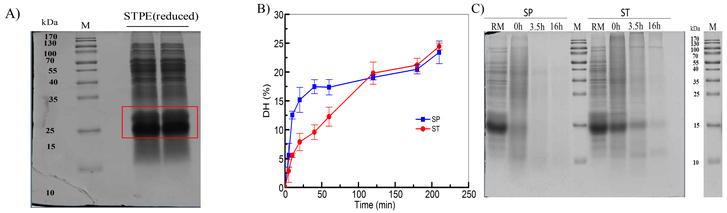
INTRODUCTION

Spirulina, a blue green microalgae belonging to cyanobacteria family, is considered as promising unconventional food due to its high content of protein (60-70% of dry biomass). [1] Among various microalgae, spirulina is considered by World Health Organization as “one of the greatest superfood on the globe” [1], not only for its abundance and balanced amino acid composition but also for it produces bioactive peptides with spectrum pharmaceutical properties such as antioxidant, anticancer, antiflammatory, antihypertensive, immunomodulatory effects, reported in some *in vitro* and *in vivo* studies. However, even if many functional aspects have been widely investigated, the knowledge on its encrypted bioactive peptides it is still at the beginning. In particluar, **C-Pycocyanin (C-PC)**, the most abundant protein in spirulina has a great potential of research and development as a drug or functional food.

AIMS

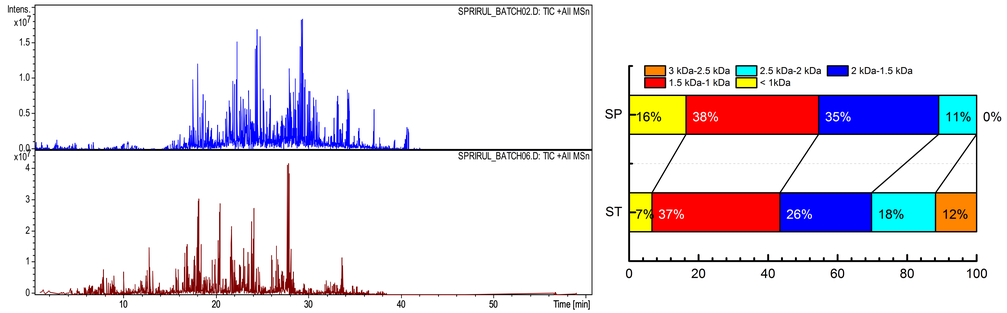
Considering the good development prospect and high content of C-PC (up to 10–20%) in *S. platensis* [2], we proposed a **high-throughput peptidomic strategy** to get insight into peptide expression derived from tryptic and peptic hydrolysis of S. platensis proteins. The isolation and purification, the screening of **DPP-IV inhibitory activity** of both hydrolysates and **C-PC bioactive derived peptides** was here applied providing a relevant basis for the development of natural product useful in the diabetes treatment and preventions.

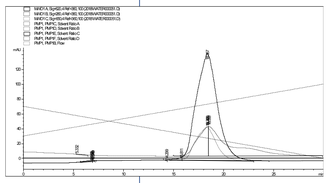
RESULTS



- *Spirulina* proteins were hydrolyzed by pepsin (SP) and trypsin (ST), respectively. Fig 2 B showed the degree of hydrolysis (DH%) detected by o-phthaldialdehyde (OPA) assay. During the first 3.5 h, more than half of hydrolysis was performed with DH of 23.4% and 24.4% for ST and SP, respectively. And the maximum rate of hydrolysis for both trypsin and pepsin was achieved during the first 30 min. After digestion overnight, ST group showed higher DH (49.4%) than SP group (37.8%).

Figure 2. A) Proteomic SDS-PAGE profile of total protein extract from *Spirulina* (STPE); Hydrolysis of total *Spirulina* protein. **B)** Determination of DH (%) at different time point of protein hydrolysis during first 3.5 h of digestion, **C)** SDS-PAGE analysis of hydrolysates from SP and ST group at different hydrolysis time points.





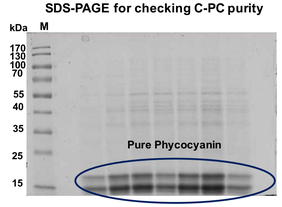
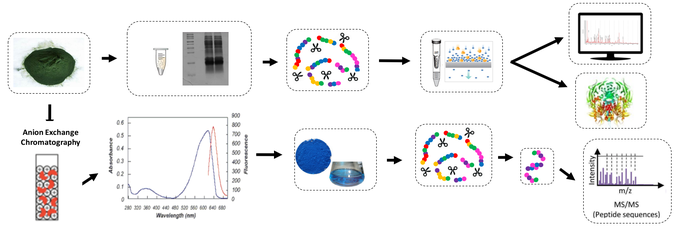


Figure 3. A) HPLC-MS/MS profile of tryptic (blue) and peptic (red) hydrolysates. **B)** Molecular weight distribution of peptides identified from hydrolysate fraction less than 3 kDa in SP and ST group. **C)**

MATERIAL AND METHODS

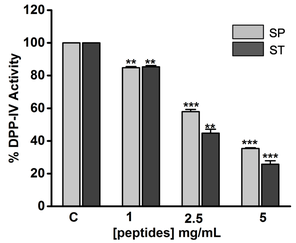


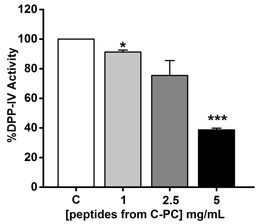
Ultrasound-Assisted Protein Extraction from Spirulina: 0.5 g of Spirulina defatted powder were suspended in 10 mL of NH_4Cl solution (0.05 M, pH 4.39). The mixture was treated with ultrasonic cell disruptor for 6 min, conducted as 5s at 50 W, 23kHz frequency pulses followed by 5s of cool-down period in ice.

C-Phycocyanin purification: The first precipitation 25% (w/v) of $(NH_4)_2SO_4$. The pellet was recovered through centrifugation at 10000 rmp at 4℃ for 30 min and solubilized in 5 mL of 0.1 M of sodium phosphate buffer, pH 7.8. The precipitated C-PC was loaded to DEAE-Sephadex column and eluted with NaCl solution of linearly increasing ionic concentration from 0.3 to 1 M at a flow rate of 1 ml/min

Figure 1. Workflow adopted for protein extraction, C-PC purification, hydrolysis, data analysis and DPP-IV activity evaluation

In vitro Biochemical Evaluation of the Inhibition of DPP-IV Activity





[1] Ruma Arora Soni, K. Sudhakar, R.S. Rana. *Trends in Food Science & Technology*, 69, pp. 157-171 (2017); [2] Minic S L, Stanicvucinic D, Vesic J, et al. Journal of Proteomics, 2016, 147:132-139